# SEAMAP
# SOUTHEAST FISHERIES SCIENCE CENTER
# ZOOPLANKTON Sorting PROTOCOLS

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The primary objective of SEAMAP/SEFSC zooplankton analyses is to build a database on the abundance of commercially important decapod crustacean larvae in the Gulf of Mexico and to assemble a collection of specimens for further taxonomic analysis. This work was initiated by SEAMAP/SEFSC under the guidance of Dr. Ken Stuck of the Gulf Coast Research Laboratory in the late 1980's. A more recent, secondary objective is to identify and count the other major zooplankton components of SEAMAP samples and to assemble a collection of specimens that can be used as 'reference/training' specimens for use with ZOOSCAN to develop automated image recognition routines.

GENERAL INSTRUCTIONS:

Decapods (see list below) are to be sorted **(in distilled water)**, removed and placed in individual labeled vials with **95% ethanol** from every sample. **The aliquot to be sorted and the taxa to be removed will depend on the plankton gear/net used to collect the sample. Decapod Protocols A and B explain the separate requirements for each type of sample depending on gear.**

All 'Other Zooplankton' specimens are to be sorted **(in distilled water)**, removed and placed in a single labeled vial with **95% ethanol** from every sample. Individual taxa are no longer to be placed in separate vials.

Separate data sheets, one for the decapods and the other for the remaining zooplankton taxa are to be filled out. These data sheets are to be copied with one copy being sent to Dr. Joanne Lyczkowski-Shultz, Mississippi Labs, Pascagoula, MS. and the original to be kept at ZSIOP, Szczecin for future reference.

The vials containing all specimens are to be shipped to Dr. Joanne Lyczkowski-Shultz, Mississippi Labs, Pascagoula, MS.

When fish eggs and/or larvae are found in a sample during zooplankton analysis the entire sample must be resorted for fish eggs and larvae. Specimens of fish eggs and larvae must be removed and given to the Ichthyoplankton Chief for identification. ISR and IDRL data sheets for these fish eggs and larvae along with the vials containing the specimens should be included with the shipment of zooplankton specimens to Dr. Joanne Lyczkowski-Shultz.

Protocol A: DECAPOD CRUSTACEAN - BONGO and MOCNESS SAMPLES:

Taxa to be sorted from BONGO and MOCNESS samples:

1. Lobster phyllosoma and **puerulus** (list separate counts on data sheet)
2. Penaeidae postlarvae
3. Portunidae megalopae
4. Sicyoniidae postlarvae
5. *Menippe* megalopae
6. Geryonidae megalopae

7. Penaeidae larvae
8. Portunidae zoeae
9. Sicyoniidae larvae
10. Geryonidae zoeae
11. *Menippe* zoeae
12. **Caridean shrimp larvae**
13. **Stenopodid shrimp larvae**
14. Solenoceridae and **other Penaeidae larvae**
15. **Anomuran crab larvae**
16. **Sergestidae**
17. **Luciferidae**

18. **Other and damaged Decapods (larvae)**

Sorting Procedure for BONGO and MOCNESS samples:

1. Measure displacement volume of the sample OR use the previous measurement of displacement volume when the sample was sorted for ichthyoplankton. This previous measurement can be found on the ISR sheet or data file. Record the required sample collection information and the displacement volume on SEAMAP/SEFSC Decapod Crustacean Larvae Data Sheet 1.

2. The sample should be split using a Folsom or comparable plankton splitter until an aliquot containing approximately 200 to 400 decapod larvae **(taxa 7 to 17)** is obtained. When splitting the sample each split should be placed in individual beakers. In most cases, samples should be split to obtain a final aliquot size of 1/64. If the total number of larvae of taxa **7-17** (from above list) removed from the smallest aliquot (one of the final pair) is less than 200, the remaining aliquot from the final pair should also be sorted. If necessary, additional aliquots should be sorted until a minimum of 200 larvae of taxa **7-17** have been obtained.

3. When a minimum of 200 larvae have been obtained, remove specimens of **taxa 7-17** and place them in individual labeled vials. The number of specimens removed should be recorded on the data sheet together with the final aliquot sorted for each taxon. The final aliquot size sorted for each taxon should be calculated by the addition of all the sample fractions sorted. For example: if both 1/64 fractions and the 1/32 fraction were sorted to obtain the minimum of 200 larvae then the final aliquot recorded on the data sheet should be 1/16.

4. If displacement volume of the sample is 20 ml or less, the (entire) remainder of the sample should be sorted for **taxa 1-6 and 18** (from above list). If the displacement volume is greater than 20 ml, the portion of the sample to be sorted for **taxa 1-6 and 18** should be determined using the following schedule:

| Displacement volume | Aliquot to be sorted |
|---|---|
| 21-40 ml | 1/2 |
| 41-80 ml | 1/4 |
| 81 ml or greater | 1/8 |

5. When the required portion of the sample is sorted for larvae of **taxa 1-6 and 18** the vials containing those taxa should be individually labeled and sealed. The number of specimens sorted should be recorded on the data sheet together with the final aliquot (portion) sorted for each taxon. All specimens are to be placed in labeled vials containing **95% ethanol** (use labels supplied by Pascagoula for ichthyoplankton).

Protocol B: DECAPOD CRUSTACEAN - NEUSTON SAMPLES

Taxa to be sorted from NEUSTON samples:

1. Lobster phyllosoma (all species).
2. Penaeidae postlarvae
3. Portunidae megalopae
4. Sicyoniidae postlarvae
5. *Menippe* megalopae
6. Geryonidae megalopae

18. Other and damaged Decapods (postlarvae and megalopae**).**
**Sergestidae and Luciferidae are NOT to be removed or counted from neuston samples.**

Sorting Procedure for NEUSTON samples:

1. Measure displacement volume of the sample after removing debris, *Sargassum* etc. Record the required sample collection information and the displacement volume on SEAMAP/SEFSC Decapod Crustacean Larvae Data Sheet 1.

2. If displacement volume of the neuston sample is 30 ml or less, the entire sample should be sorted for **taxa 1-6 and 18** (from above list) only. If displacement volume is greater than 30 ml, the portion of the sample to be sorted should be determined using the following schedule:

| Displacement volume | Aliquot to be sorted |
|---|---|
| 31-60 ml | 1/2 |
| 61-120 ml | 1/4 |
| 121-240 ml | 1/8 |
| 241 ml or greater | 1/16 |

   If a large portion of the sample consists of *Sargassum* or coelenterates, a larger aliquot should be sorted.

3. When the required portion of the sample is sorted for larvae of **taxa 1-6 and 18** the vials containing those taxa should be individually labeled and sealed. The number of specimens sorted should be recorded on the data sheet together with the final aliquot (portion) sorted for each taxon. All specimens are to be placed in labeled vials containing **95% ethanol** (use labels supplied by Pascagoula for ichthyoplankton).

Protocol C: OTHER ZOOPLANKTON - BONGO and NEUSTON samples

Taxa of zooplankton to be sorted are listed on SEAMAP/SEFSC Other Zooplankton Data Sheet 2.

Sorting Procedure for bongo and neuston samples:

1. The sample should be split using a Folsom or comparable plankton splitter until an aliquot containing approximately 400-500 zooplankters is obtained. Stir/ bubble the sample, and take an aliquot using a Stempel Pipette or Folsom plankton splitter. The ideal aliquot should be enough to count at least 200 copepods and 200 of everything else combined, but small enough to prevent going over 500 for either.

2. Identify the organisms to the taxonomic level indicated on SEAMAP/SEFSC Zooplankton Data Sheet 2.

3. **When heads and tails are present (most common for Larvaceans and Chaetognaths) count only the heads and add that number to the count of entire specimens. The total count for that taxon to be recorded on the data sheet is the number of entire specimens + the number of heads.**

4. When the required portion of the sample is sorted for the listed zooplankton the vials containing those taxa should be individually labeled and sealed. The number of specimens sorted should be recorded on the data sheet together with the final aliquot (portion) sorted for each taxon. All specimens are to be placed in labeled vials containing 70% ethanol (use labels supplied by Pascagoula for ichthyoplankton).